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OIL AND MUCILAGE CELLS IN ANNONA (ANNONACEAE) AND THEIR SYSTEMATIC SIGNIFICANCE

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SUMMARY

The morphology and distribution patterns of oil and/or mucilage cells, i.e. idioblasts, in the leaf of 37 *Annona* species are described. Idioblasts are always present in the spongy parenchyma in all species and in most cases also in the palisade parenchyma. Usually both oil cells and mucilage cells occur; in some species either oil or mucilage idioblasts are present. Their number ranges from few to abundant. Both idioblast types possess a suberized wall layer. Mucilage cells are significantly larger than oil cells. The distribution patterns of oil and/or mucilage cells often do not coincide with the current classification into sections. Leaf features such as lamina thickness, the presence of sclereids in the mesophyll, crystals in the adaxial epidermis, hairs, an adaxial hypodermis, a thickened cuticle and the presence of a papillate abaxial epidermis and sunken stomata are compared with the distribution of oil and mucilage cells. Following an analysis of the systematic value of oil and mucilage cells it is concluded that combinations and frequencies of oil and mucilage cells are often useful in species identification, and sometimes indicate relationships between species. In general, the significance of oil and mucilage cells as phylogenetic markers is extremely limited as shown in tentative cladistic analyses of leaf anatomical characters.

INTRODUCTION

In previous studies we have provided developmental and ultrastructural evidence for homology of oil and mucilage cells in the genera *Cinnamomum* and *Annona* (Bakker & Gerritsen, 1989, 1990; Bakker et al., 1991). In the present paper the specific and systematic value of the distribution of both types of idioblasts at and below the genus level will be explored for *Annona*.

Annona is a largely neotropical genus with approximately 125 species (Safford, 1914; Fries, 1931, 1959; Maas et al., 1987). Some species occur in tropical Africa (Beyer, 1902; Robyns & Ghesquière, 1934) and Asia (Jovet-Ast, 1942; Sinclair, 1955). The genus is especially known for its edible fruits (George & Nissen, 1991; Koesriharti, 1991).

The macromorphology of Annonaceae has been extensively studied and classifications of *Annona* are mainly based on the floral morphology and fruit/seed characteristics (e.g. Engler & Diels, 1900; Safford, 1911, 1914; Fries, 1931, 1943, 1959). Leaf anatomical characters were briefly described by Beyer (1902), Robyns & Ghesquière (1934) and more extensively by Klucking (1986), Roth (1981) and Van Setten & Koek-Noorman (1986).

Table 1. List of material studied of *Annona*.
(L = Rijksherbarium Leiden; U = Institute for Plant Systematics Utrecht;
h = herbarium; a = alcohol collection; c = cultivation)

acutiflora Mart.		*Maas et al. 7097*	Brazil	(U-a)
amazonica R.E. Fries		*Daly et al. 3954*	Brazil	(U-h)
ambotay Aubl.		*Maas et al. 6695*	Brazil	(U-a)
bicolor Urb.:	1)	*Fuertes 258*	Dominic. Rep.	(L-h)
	2)	*Zanoni 35322*	Dominic. Rep.	(U-a)
cacans Warm.		*Silva 90*	Brazil	(U-h)
cherimola Mill.:	1)	*Karthikeyan 26802*	Asia	(L-h)
	2)	Hort. Bot. Utrecht *81GR00133*	Peru	(c)
chrysophylla Boj.		*Hildebrandt 1253*	Tanzania	(L-h)
cordifolia R.E. Fries		*Maas et al. 5990*	Peru	(U-a)
deminuta R.E.Fries		*Steward & Ramos P17660*	Amazone Area	(U-h)
diversifolia Safford		*Lopez Forment 1116*	Mexico	(L-h)
duckei Diels:	1)	*Brandbyge et al. 33458*	Ecuador	(U-h)
	2)	*Berg & Akkermans 1058*	Ecuador	(U-a)
dumetorum R.E. Fries		*Maas & Zanoni 6396*	Dominic. Rep.	(U-h/a)
foetida Mart.		*Daly et al. 1183*	Brazil	(U-h)
glabra L.:	1)	Curtiss 502	Cuba	(L-h)
	2)	Hort. Bot. Utrecht *83GR00360*	Florida	(c)
glauca Schumacher & Thonn.		*Leprieur s.n.*	Senegal	(L-h)
globiflora Schlechtend		*Purpus 2443*	Mexico	(L-h)
gracilis R.E. Fries		*Ekman H8745*	Haiti	(U-h)
haitiensis R.E. Fries		*Ekman H14855*	Dominic. Rep.	(U-h)
hayesii Safford		*Miller 1022*	Panama Can. Zone	(U-h)
hypoglauca Mart.		*Maas et al. 7408*	Guyana	(U-h)
longiflora S. Watson		*Pringle 9651*	Mexico	(L-h)
cf. *macrocalyx* R.E. Fries		*Maas et al. 6211*	Peru	(U-a)
montana Macfad.:	1)	*L. 000*	C/S America	(L-h)
	2)	Bogor Bot. Garden *XXD42a*	Java	(L-h)
	3)	*Lanjouw & Lindeman 1373*	Suriname	(U-a)
	4)	Hort. Bot. Utrecht *76GR00116*	C/S America	(c)
muricata L.:	1)	*Keith A1516*	Borneo	(L-h)
	2)	*Thompson 275*	Mariane Islands	(L-h)
	3)	*Splitgerber s.n.*	C/S America	(L-h)
	4)	Hort. Bot. Utrecht *87GR0002*	Cameroon	(c)
nutans R.E. Fries		*Hassler 12355*	Paraguay	(L-h)
phaeoclados Mart.		*Dubs 445*	Brazil	(U-h)
pittieri Donn. Sm.		*Zamora & Carlson 428*	Costa Rica	(U-h)
purpurea Moc. & Sess		Hort. Bot. Utrecht *79GR00026*	Mexico	(c)
reticulata L.:	1)	*Powell & H'ng Kim Chey 254*	Java	(L-h)
	2)	*Broadway s.n.*	Grenada (Am.)	(L-h)
scandens Diels		*Maas et al. 6029*	Peru	(U-a)
senegalensis Pers.:	1)	*Jacques-Félix 3388*	Cameroon	(L-h)
	2)	*Berg 151*	Togo	(U-h)
sericea Dunal:	1)	*?, s.n.*	s.l.	(L-h)
	2)	*Maas et al. 7144*	Guyana	(U-h)
	3)	*Prance 25302*	Brazil	(U-a)
spraguei Safford		*Hamilton 576*	Panama	(U-h)
squamosa L.:	1)	*Perianayogam et al. RHT 23777*	Asia	(L-h)
	2)	*Kooy 632*	Little Sunda Islands	(L-h)
	3)	*Bosla 13*	Asia	(L-h)
	4)	*Hort. Bot. Utrecht 82GR00235*	Burundi Antilles	(c)
tenuiflora Mart.		*Steyermark s.n.*	Venezuela	(U-h)
spec.		*Maas et al. 6186*	Peru	(U-h)
spec. nov.		*De Granville et al. 10907*	French Guiana	(U-h)

The multidisciplinary project on the Annonaceae (Maas, 1984; Koek-Noorman, 1987) was initiated to collect as many data as possible to provide a solid base for a natural classification. The present study deals with the oil and mucilage cells in the leaves of *Annona* species. The main part includes aspects of oil and mucilage cells: morphology and distribution patterns. In addition some other leaf anatomical features are described and compared with oil/mucilage cell distribution patterns. These features are compared with Fries' classification (1931, 1959) of *Annona*. Tentative cladistic analyses of the leaf anatomical features, including the oil and mucilage cells, were carried out in order to explore the potential value of oil and mucilage cells as phylogenetic markers (synapomorphies).

MATERIAL AND METHODS

Material and fixation

The 54 specimens of the 37 studied species of *Annona* are listed in table 1. Differently prepared leaf material was used in this study.

FAPA fixation — Leaves from herbarium vouchers, kept in Utrecht (U-h, table 1) and Leiden (L-h, table 1), were boiled in water until permanent sinking of the leaf in cold water and then fixed/stored in FAPA-solution. Leaves stored in alcohol (96%) in Utrecht (U-a, table 1) were transferred into FAPA.

Fresh leaves of *A. muricata, A. squamosa, A. cherimola, A. purpurea, A. montana,* and *A. glabra,* grown in the tropical greenhouse in the Hortus Botanicus at Utrecht (c, table 1) were removed from the cultivated plants and immediately fixed in FAPA.

Karnovsky / OsO_4 fixation — Fresh leaves and shoot apices from the species mentioned above were cut into small pieces and lengthwise respectively, fixed in modified Karnovsky fixative followed by OsO_4, and finally embedded in Epon (see Bakker & Gerritsen, 1989).

Sectioning, staining procedures, and microscopy

1) From the FAPA-fixed leaves transverse sections of the central part of the lamina were made at 30 µm thickness for light microscopical examination

   a) Sections were cut in alcohol 96% to prevent dissolution of the mucilage. After bleaching these sections were stained in Alcian Blue (1% in alcohol 70%; Richter, 1977), dehydrated and mounted in Depex.
   Sections used as control samples for the mucilage staining were sectioned in demineralized water and further processed as described above. In these sections mucilage will have flowed out of the cells in the presence of water. Therefore the control-samples should be negative for mucilage staining.

   b) Sections were cut in demineralized water to prevent dissolution of oil. After bleaching sections were stained for oil with Chrysoidin/acridin red (0.5%/ 0.5% in water; Richter, 1977) or in Sudan IV (1% in alcohol 70%), washed in water and finally mounted in glycerine jelly.

c) Sections were cut in demineralized water and, after bleaching, stained in Berberine-hemisulphate (1% in water), followed by Anilin Blue (1% in water) for the detection of suberin (Brundrett et al., 1988), and mounted in glycerine jelly. The sections were examined with a fluorescence microscope using a DAPI-A filter.
d) Sections were cut in demineralized water and mounted unstained in glycerine jelly for autofluorescence (of suberin).

2) Fresh leaves and shoot apices embedded in Epon.
a) Sections of 1 μm thickness were stained with Toluidine Blue O for overall staining of the tissue at light microscopical level.
b) Ultrathin sections were stained with uranyl acetate and lead citrate for examination with the transmission electron microscope.

Examination, measurements, and analysis

In the 30 μm thick transverse sections stained for oil and/or mucilage the number of oil and/or mucilage cells present in the palisade and/or spongy parenchyma were counted and calculated per mm leaf width. The oil and mucilage cells were measured for their height and width. As a rough measure for the size of the cells the height and width were averaged. The height was divided by the width to obtain an index for the shape of the cells. In the same sections lamina thickness was measured halfway between the midvein and the margin and the number of hairs was counted per mm leaf width. The density and position of the hairs was confirmed by examination of entire leaves with a stereo-microscope and a scanning electron microscope. For a tentative cladistic analysis of the leaf anatomical diversity the computerprogram HENNIG86 version 1.5 (Farris, 1989) was used.

RESULTS

General leaf anatomy

The general leaf anatomical description of *Annona* presented below is based on original observations in combination with data from the literature (Van Setten & Koek-Noorman, 1986).

The lamina is mostly dorsiventral, sometimes isobilateral. Uniseriate, 2–4-celled, non-glandular trichomes are present on the abaxial side and in many cases also on the adaxial side of the leaf. The appressed (mostly thin-walled) or erect (mostly thick-walled) trichomes occur either singly, in most species with a length of 100–600 μm and an acute top cell, or as small tufts of 2 (3 or 4) hairs (few species). Few species do not possess hairs at all. In some species an adaxial hypodermis is present. In other species the adaxial epidermis is covered by a relatively thick cuticle. The cells of the spongy parenchyma are sometimes elongated parallel to the epidermis and the palisade parenchyma may consist of 2 or 3 layers. Globular oil and/or mucilage cells are always present in the spongy parenchyma (oil cells: 35 × 35 μm; mucilage cells: 46 × 45 μm). In most species ellipsoid oil and/or mucilage cells are also present in the palisade parenchyma (oil cells: 33 × 36 μm; mucilage cells: 40 × 51 μm). In part of the species filiform sclereids are present in the mesophyll. Druse crystals in the adaxial epidermis are very common to absent and sometimes rhombic crystals occur.

In the abaxial epidermis crystals appear in lower frequencies and are sometimes also located in the mesophyll surrounding the veins. The vascular system of the midvein consists of a collateral abaxial arc and adaxial plate, sheathed by sclerenchymatous fibres. The lower order veins are embedded in the mesophyll. Sporadically the veins may be vertically transcurrent by indistinct bundle sheath extensions.

Oil and mucilage cells

By combining the different light microscopical staining reactions it was possible to discriminate between mucilage and oil cells.

Sudan IV – The contents of oil cells usually stained red/pink to dark-red or brown-red and were present as a mass filling the whole cell-lumen (fig. 1A) or as an oildrop whether or not visibly attached to the wall by a cupule (fig. 1B). The suberized wall layer could be distinguished by its red colour. Empty cells present in the palisade and spongy parenchyma in most cases were mucilage cells. Empty oil cells could be recognized by their thick (swollen) cell wall (fig. 1B) and the ring-like appearance of the cytoplasm.

Chrysoidin/acridin red – In most cases the whole leaf section was stained red. The oil cells with or without contents could be distinguished by the similar appearance as with Sudan-staining. Mucilage cells were unstained (empty) or sometimes appeared light-yellowish.

Alcian Blue – Both in alcohol and water sections the whole tissue of herbarium as well as fresh leaves stained blue. The oil cells stained glossy greenish-blue and could be distinguished from the mucilage cells by their thick dark blue cell wall, which is indistinct in mucilage cells, and the ring-like appearance of the cytoplasm (fig. 1C). In the alcohol sections the retained mucilage was stained distinctly and sometimes was slightly swollen resulting in extrusion from the mucilage idioblasts. The mucilage often showed some striation radiating from the centre (fig. 1D). In the control samples the mucilage cells stained lightly or remained unstained depending on the amount of dissolved mucilage.

In alcohol sections of alcohol-collected leaves the mucilage did show little coloration after staining with Alcian Blue, but could be recognized by the glossy appearance of the mucilage in the cells (fig. 1E). The cytoplasm embedded in the mucilage was recognizable as strands reaching towards the cell wall. The inability of the mucilage to stain is presumably an effect of the prolonged alcohol fixation, prohibiting the penetration of the stain. This phenomenon is also present in sections of leaves which have been kept in FAPA for a long period. In the control sections of alcohol-collected leaves the distinctly blue-coloured mucilage spread out over the section, thereby often obscuring the mesophyll. Presumably, the phenomenon is an effect of water, in which the mucilage finally started to swell and partly dissolved, whereby the stainability for Alcian Blue returned.

Fluorescence – The suberized wall layer showed autofluorescence in the cell wall of both types of idioblasts. In the sections stained specifically for suberin (berberin) this layer appeared in the oil and mucilage cells as a light yellow/whitish small line in the cell wall; earlier described for oil cells of *Annona muricata* (Bakker & Gerritsen, 1990).

1 µm sections – Oil cells were easily recognized by their overall cytoplasmic composition (fig. 2A) and/or the presence of a cupule (fig. 2A, B). Mucilage cells were recognized by their characteristic cytoplasmic strands and/or the distinctly purple stained mucilage (fig. 2F, H). Mucilage can break through the wall and fill the lumina of surrounding cells and intercellular spaces in the leaf, especially the spongy parenchyma (e.g. in *Annona squamosa:* fig. 2H), or intrude a neighbouring non-mucilage cell.

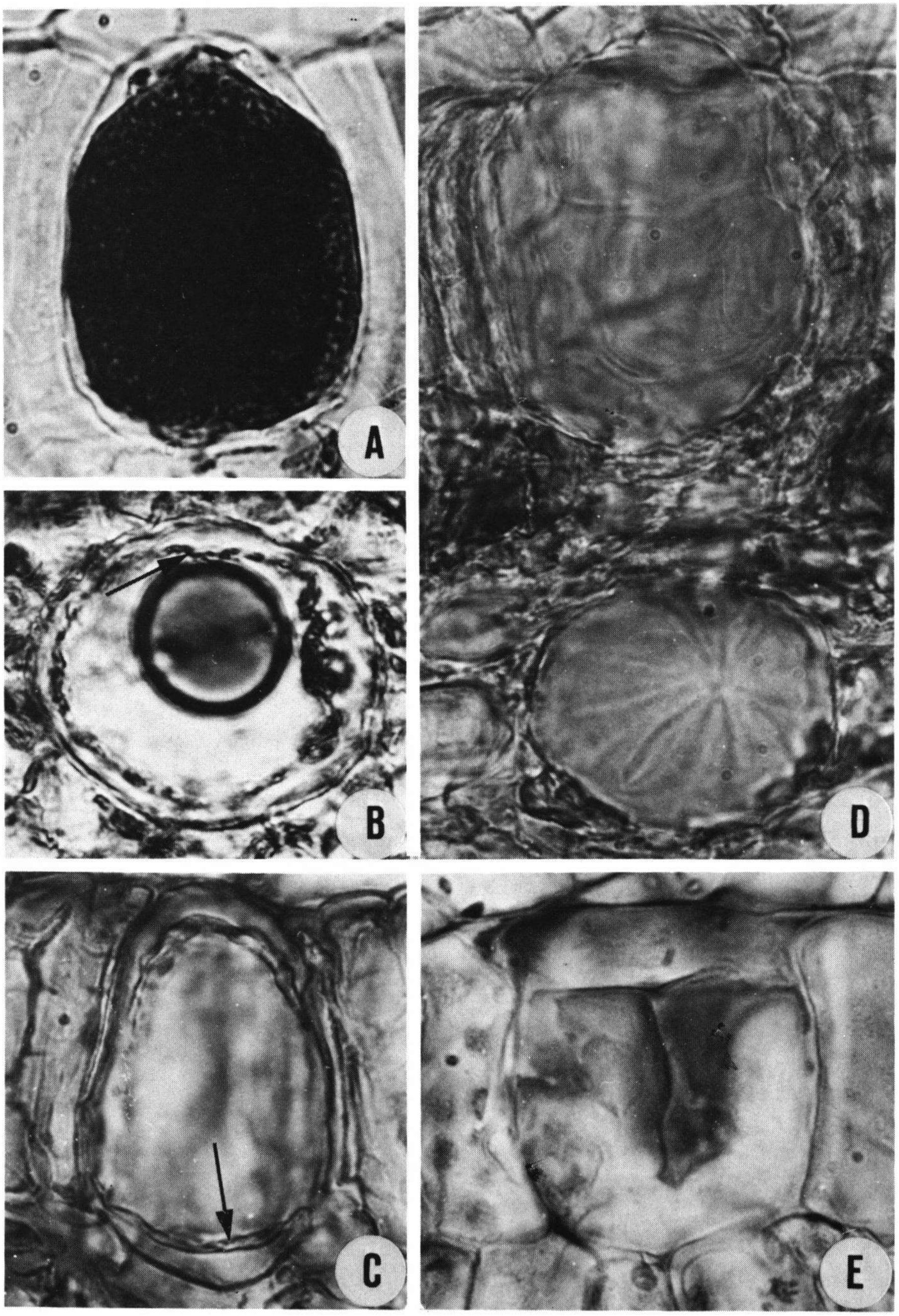
Transmission electron microscopy – At the ultrastructural level a suberized layer was observed in both oil (fig. 2D, E) and mucilage cells (fig. 2G). In the young stages of development of the idioblasts the suberized layer showed lamellations (fig. 2D) which disappeared in later stages (fig. 2E, G). In older developmental stages an inner wall layer was always present in oil cells (fig. 2E). Cupules of varying sizes were detected in the oil cells (shown for *Annona glabra*; fig. 2C). Details of oil cells in *Annona muricata* have been described in a previous report (Bakker & Gerritsen, 1990).

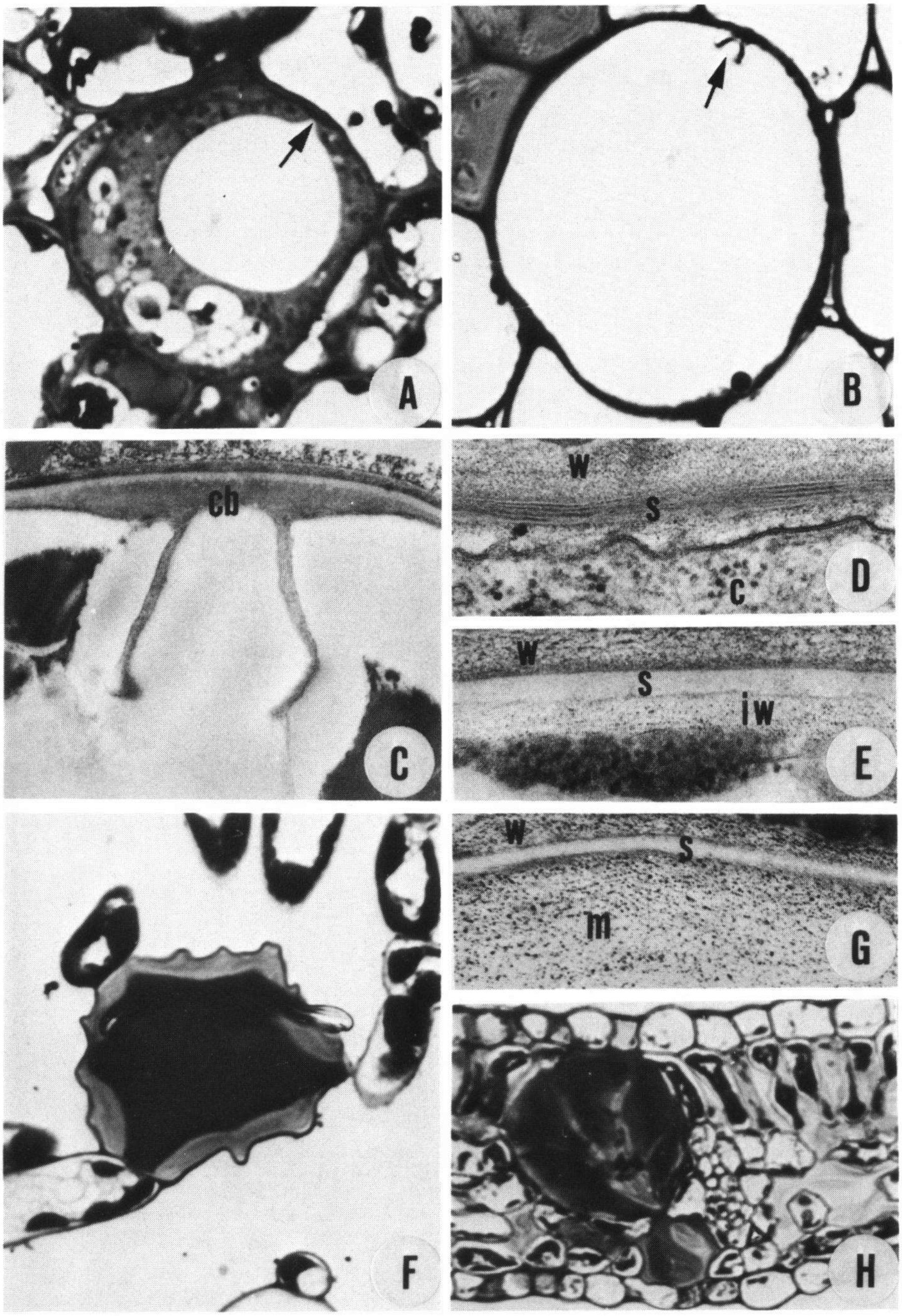
Distribution

The distribution (absence/presence) of oil and mucilage cells in the palisade and spongy parenchyma, which varies considerably, is described below. In case an idioblast was located in the transition zone between the palisade and spongy parenchyma its position was assigned to the spongy parenchyma.

Combinations – In theory 16 combinations of oil and mucilage cell distributions in the palisade and spongy parenchyma are possible. However, the four combinations lacking idioblasts (oil and/or mucilage cells) in the spongy parenchyma do not occur. Idioblasts are always present in the spongy parenchyma; either exclusively as oil or mucilage cells, or both in combination (tables 2 and 5). The combination which exclusively showed oil cells in the palisade parenchyma or in both the palisade and spongy parenchyma occurred most frequently. The combinations in which only mucilage cells were present in the mesophyll occurred in three species. The other combinations (oil and/or mucilage cells in palisade and spongy parenchyma) were found in the remainder of the species.

Fig. 1. Transverse 30 µm sections of *Annona* species (LM). A, B: stained in Sudan IV; C–E: stained with Alcian Blue. — A: *A. sericea* 1. Oil cell in palisade parenchyma. Oil fills the cell lumen; × 850. — B: *A. glabra* 1. Oil cell in spongy parenchyma. Oil drop seems to be attached to the upper cell wall by a cupule (arrow). Note the swollen cell wall; × 850. — C: *A. dumetorum*. Oil cell in palisade parenchyma. Note the glossy appearance of the cell and the ring-like cytoplasm (arrow); × 850. — D: *A. senegalensis* 2. Mucilage cells in palisade and spongy parenchyma. Note the radiating striation in the stained mucilage of the lower cell; × 740. — E: *A. acutiflora*. Mucilage cell in palisade parenchyma. Note the typical appearance of the mucilage when fixed in alcohol and/or stored for a long time in FAPA. The mucilage is unstained. The centrally located cytoplasm is stained distinctly; × 850.


A
B
C
D
E


A
B
cb
C
w
s
c
D
w
s
iw
E
w
s
m
G
F
H

Eleven species of which more than one specimen was studied (table 1) mostly were constant for the kind of combination in which oil and/or mucilage cells occur (e.g. *Annona montana* and *A. muricata*). However, four of these species (*A. bicolor, A. senegalensis, A. sericea,* and *A. squamosa*) showed (slightly) different distribution patterns of oil and/or mucilage cells. For practical reasons these specimens have been treated as separate entities throughout this study.

Frequency – The number of oil and/or mucilage cells was assigned to various frequency classes (table 2). There are no species without any idioblasts in the leaves. In almost all species (93%) oil cells are present (tables 2 and 5). There are many species without mucilage cells (c. 46%), but when mucilage cells are present in the mesophyll a higher amount was observed than for oil cells in most cases.

The frequency of idioblasts in the lamina of different species was highly variable: approximately 0.4 cells/mm leaf width (*Annona deminuta*) to over 25 cells/mm leaf width (*Annona* spec.).

Size and shape

The values for the size and shape of oil and mucilage cells are given in table 3. Any effect on cell size of different fixation procedures was not apparent.

In the palisade parenchyma the oil and mucilage cells are mostly ovoid (height/width ratio > 1.1; fig. 1A, C, D, E); in spongy parenchyma the cells are more globular (ratio 1.0) in shape (fig. 1B, D; table 3).

The mucilage cells in most cases are larger than oil cells in the same mesophyll layer (except in the spongy parenchyma of *Annona sericea*, table 3). The difference in size between oil and mucilage cells appeared to be significant (Chi-square test, $P < 0.05$).

Fig. 2. Epon embedded leaves of *Annona*. A, B, F, H: 1 μm sections stained with Toluidine Blue (LM); C–E & G: Ultrathin sections stained with uranyl acetate and lead citrate (TEM). — A: *A. montana* 4. Young oil cell in the shoot apex. Note the empty oil cavity attached to the cell wall by a cupule (arrow); × 1220. — B: *A. glabra* 2. Empty oil cell in the apex showing a large cupule (arrow); × 850. — C: *A. glabra* 2. Detail of a cupule in an oil cell in the apex. Note the cupule-base (cb), i.e. the thickened part of the inner wall layer; × 11,500. — D: *A. montana* 4. Detail of the cell wall of a young oil cell in the apex. Note the lamellations present in the suberized layer (s) deposited against the primary cell wall (w); c = cytoplasm; × 57,700. — E: *A. glabra* 2. Detail of the cell wall of an oil cell in the apex, showing the suberized layer (s) and the inner wall layer (iw) deposited against the primary cell wall (w); × 57,700. — F: *A. purpurea*. An (almost) intact mucilage cell in the spongy parenchyma in the leaf. Note the differently stained mucilage layer. The outer layer is more lightly stained than the central part; × 850. — G: *A. purpurea*. Detail of the cell wall of a mucilage cell in the apex filled with mucilage (m). Note the suberized layer (s) present near the primary cell wall (w); × 57,700. — H: *A. squamosa* 4. Large mucilage cell in the palisade parenchyma. Note the presence of mucilage in the intercellular spaces of the spongy parenchyma; × 340.

Table 2. Presence of oil and/or mucilage cells in the palisade and spongy parenchyma in leaves of *Annona* species.

Species	oil cells			mucilage cells		
	pal	spo	total	pal	spo	total
acutiflora	+	++	++	+++	+	+++
amazonica	+	+++	+++	–	–	–
ambotay	+++	++	+++	–	–	–
bicolor 1	+++	+++	++++	+	–	+
bicolor 2	++++	++++	+++++	–	–	–
cacans	–	++	++	–	–	–
cherimola 1, 2	+	++	++	–	–	–
chrysophylla	–	+++	+++	++++	–	++++
cordifolia	+	+++	+++	+++	+	+++
deminuta	+	±	+	–	–	–
diversifolia	–	++++	++++	–	–	–
duckei 1, 2	–	+	+	–	–	–
dumetorum	+++	+++	++++	++++	+	++++
foetida	+	+	+	–	–	–
glabra 1, 2	+	+	+	–	–	–
glauca	±	+	+	+++	+	+++
globiflora	–	+++	+++	–	–	–
gracilis	+	–	+	+++	++	++++
haitiensis	++	++	+++	–	–	–
hayesii	++	+++	+++	–	–	–
hypoglauca	+	++	++	–	+	+
longiflora	+	–	+	++	+++	+++
cf. *macrocalyx*	+	+++	+++	+++	–	+++
montana 1–4	+++	+++	++++	–	–	–
muricata 1–4	+++	++	+++	–	–	–
nutans	–	+	+	++++	–	++++
phaeoclados	+	+	++	–	–	–
pittieri	–	–	–	+++	+++	+++
purpurea	–	–	–	–	+++	+++
reticulata 1, 2	++	++	+++	+	+++	+++
scandens	++++	++++	+++++	+++	–	+++
senegalensis 1	++	+++	++++	+	–	+
senegalensis 2	–	–	–	++++	+++	+++++
sericea 1	+	++	+++	+++	–	+++
sericea 2	+	++	+++	+++++	–	+++++
sericea 3	±	+++	+++	+++++	++	+++++
spec.	+++	++++	++++	+++++	+++	+++++
spec. nov.	–	+++	+++	+++	–	+++
spraguei	+	++	+++	–	–	–
squamosa 1, 2, 4	++	+	+++	+	++	+++
squamosa 3	++	–	++	+	+++	+++
tenuiflora	+	+++	+++	+++	–	+++

Explanation: – = absent; ± = > 0, ≤ 0.1 cell/mm leaf width; + = > 0.1, ≤ 1 cell/mm; ++ = > 1, ≤ 2 cells/mm; +++ = > 2, ≤ 5 cells/mm; ++++ = > 5, ≤ 10 cells/mm; +++++ = > 10 cells/mm.

Table 3. Size in µm [(height + width)/2] and shape (height/width ratio) of oil and mucilage cells in leaves of *Annona* species. The ratio is presented between brackets.

Species	oil cells		mucilage cells	
	palisade	spongy	palisade	spongy
acutiflora	30.5 (1.2)	28.5 (1.0)	46.0 (1.1)	42.0 (1.1)
amazonica	42.0 (1.3)	36.0 (0.9)		
ambotay	27.5 (1.0)	28.5 (1.0)		
bicolor 1, 2	38.5 (1.1)	34.0 (0.9)	56.0 (1.1)	
cacans		24.5 (0.8)		
cherimola 1, 2	28.5 (1.4)	25.5 (1.2)		
chrysophylla		40.5 (1.0)	58.5 (2.2)	
cordifolia	37.0 (1.4)	38.0 (1.2)	40.5 (1.3)	38.0 (1.1)
deminuta	40.0 (1.2)	36.5 (1.4)		
diversifolia		27.0 (1.1)		
duckei 1, 2		28.0 (1.1)		
dumetorum	36.5 (1.4)	36.0 (1.0)	48.5 (1.3)	50.0 (0.8)
foetida	46.0 (1.0)	46.0 (0.8)		
glabra 1, 2	46.0 (1.3)	40.5 (1.1)		
glauca	43.5 (1.8)	37.0 (1.0)	62.0 (1.6)	54.5 (0.9)
globiflora		30.5 (0.7)		
gracilis	43.5 (1.2)		46.5 (1.7)	38.5 (0.9)
haitiensis	44.0 (1.0)	39.5 (0.9)		
hayesii	28.0 (1.2)	27.0 (0.9)		
hypoglauca	33.0 (1.8)	29.5 (1.2)		39.5 (1.6)
longiflora	28.5 (0.9)		40.5 (1.0)	46.5 (1.3)
cf. *macrocalyx*	41.0 (1.2)	39.5 (1.2)	42.0 (1.6)	
montana 1–4	43.0 (1.2)	38.0 (0.9)		
muricata 1–4	36.0 (1.5)	32.0 (1.0)		
nutans		50.0 (1.3)	46.0 (1.4)	
phaeoclados	48.0 (1.4)	33.0 (0.9)		
pittieri			43.5 (0.8)	43.5 (0.7)
purpurea				33.0 (1.0)
reticulata 1, 2	32.0 (0.9)	35.5 (0.9)	40.0 (0.9)	45.5 (0.9)
scandens	35.0 (1.4)	35.0 (1.3)	36.0 (1.5)	
senegalensis 1	36.0 (1.0)	37.0 (1.1)	52.0 (0.9)	
senegalensis 2			51.0 (1.4)	42.0 (1.0)
sericea 1–3	38.5 (1.0)	41.0 (1.0)	39.0 (1.9)	28.0 (1.1)
spec.	33.5 (1.0)	30.5 (1.0)	48.0 (1.2)	41.0 (0.9)
spec. nov.		47.5 (1.0)	46.0 (1.7)	
spraguei	31.5 (1.7)	23.5 (1.0)		
squamosa 1–4	30.0 (1.1)	36.5 (1.0)	45.0 (1.0)	55.5 (1.0)
tenuiflora	35.0 (1.0)	33.5 (0.9)	36.0 (1.3)	

Table 4. Classification of the genus *Annona* after Fries (1959). Only the species (specimens) studied are listed. (Between brackets: total number of species included in the section or number of specimens studied per species.)

Section	(total number)	species studied
1.	*Annona* Fries (14) (*Eu-Annona* Saff. 1911)	*deminuta*
		foetida
		montana (4)
		muricata (4)
2.	*Macrantha* Fries (3)	—
3.	*Ulocarpus* Saff. (3)	*purpurea*
4.	*Campicola* Fries (2)	—
5.	*Psammogenia* Saff. (3)	—
6.	*Phelloxylon* Saff. (1)	*glabra* (2)
7.	*Helogenia* Saff.	
	Aculeato-papillosae Robyns & Ghesq. (6)	—
	Papillosae Robyns & Ghesq. (?)	*chrysophylla*
		glauca
		senegalensis (2)
8.	*Pilannona* Saff. (25)	*cordifolia*
		hypoglauca
		cf. *macrocalyx*
		scandens
		sericea (3)
		spraguei
		spec.
9.	*Gamopetalum* Saff. (7)	*nutans*
10.	*Oligantha* Fries (9)	*amazonica*
		cacans
		duckei (2)
11.	*Atractanthus* Saff. (5)	*acutiflora*
		ambotay
		hayesii
12.	*Atta* Mart (13)	*cherimola* (2)
		longiflora
		reticulata (2)
		squamosa (4)
13.	*Chelenocarpus* Saff. (4)	*pittieri*
14.	*Ilama* Saff. (2)	*diversifolia*
15.	*Saxigena* Saff. (2)	—
16.	*Annonula* Saff. (9)	*gracilis*
		haitiensis
17.	*Annonella* Baill. (5)	*bicolor* (2)
		dumetorum
		globiflora
unclassified species		*phaeoclados*
		tenuiflora
new species		spec. nov.

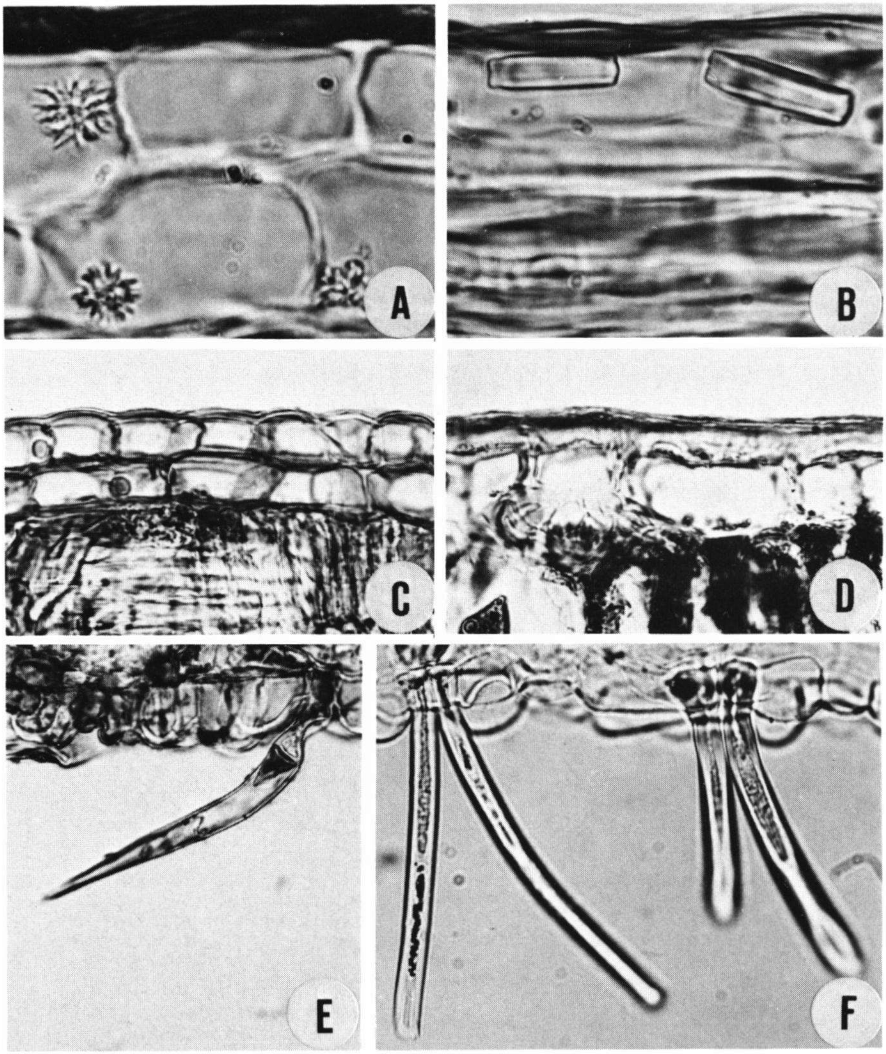


Fig. 3. Transverse 30 µm sections of leaves of *Annona* species (LM). A, B, F: Sudan IV; C, E: Alcian Blue; D: Chrysoidin/acridin red. — A: *A. nutans*. Druses present in the adaxial epidermis of the leaf; × 850. — B: *A. diversifolia*. Rhombic crystals in the adaxial epidermis of the leaf; × 850. — C: *A. nutans*. Part of the leaf showing a hypodermis underneath the adaxial epidermis; × 340. — D: *A. foetida*. Uni-layered adaxial epidermis of the leaf showing thickened outer periclinal cell walls and a thick cuticle; × 340. — E: *A. hypoglauca*. A single appressed, thin-walled, short trichome on the abaxial side of the leaf. × 340. — F: *A. sericea* 1. Double erect trichomes at the abaxial side of the leaf. Note the thick walls of the hairs; × 340.

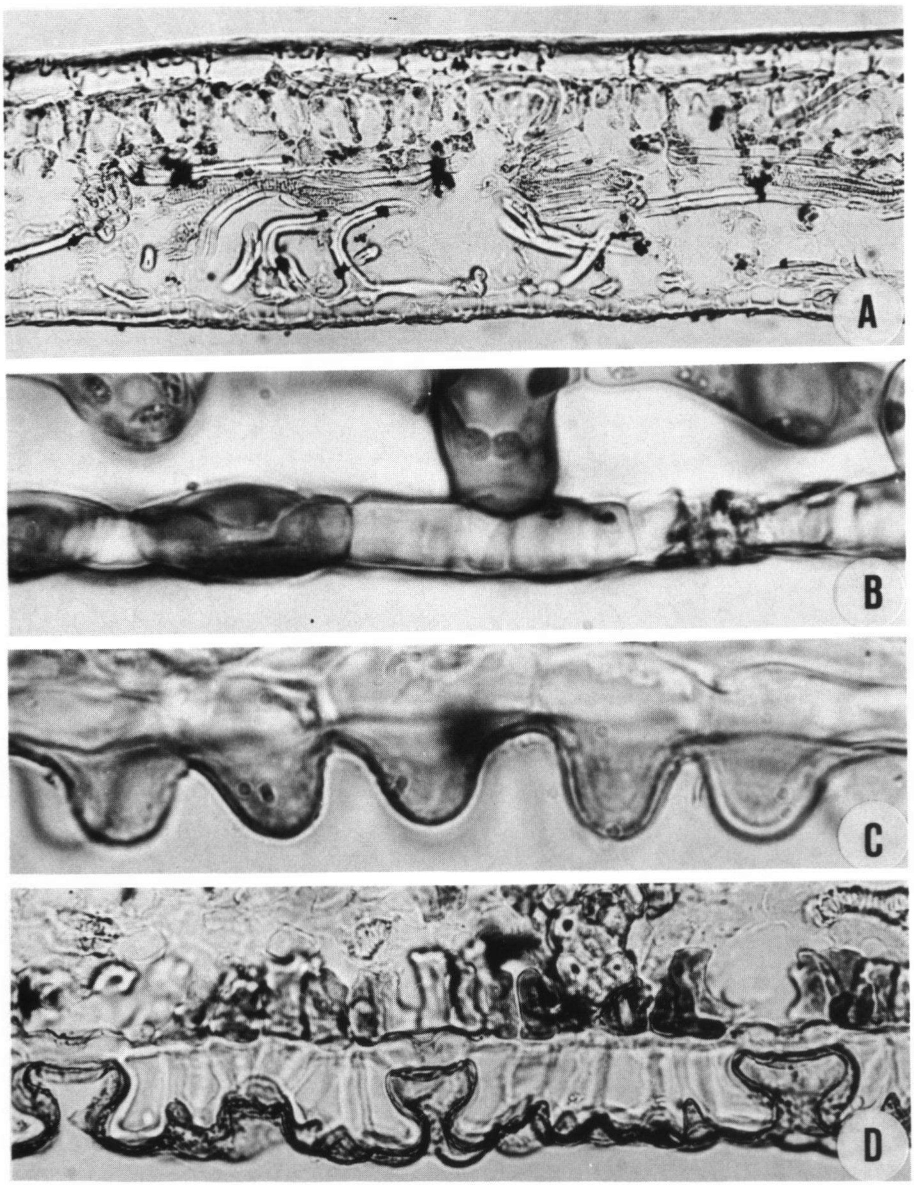


Fig. 4. Transverse 30 µm sections of *Annona* species (LM). A: Alcian Blue. B–D: Sudan IV. — A: *A. foetida*. Part of the leaf showing distinct sclereids in the mesophyll; × 140. — B: *A. acutiflora*. Flat abaxial epidermis of the leaf; × 850. — C: *A. spraguei*. Papillate abaxial epidermis of the leaf; × 850. — D: *A. nutans*. Thick abaxial epidermis of the leaf showing sunken stomata; × 340.

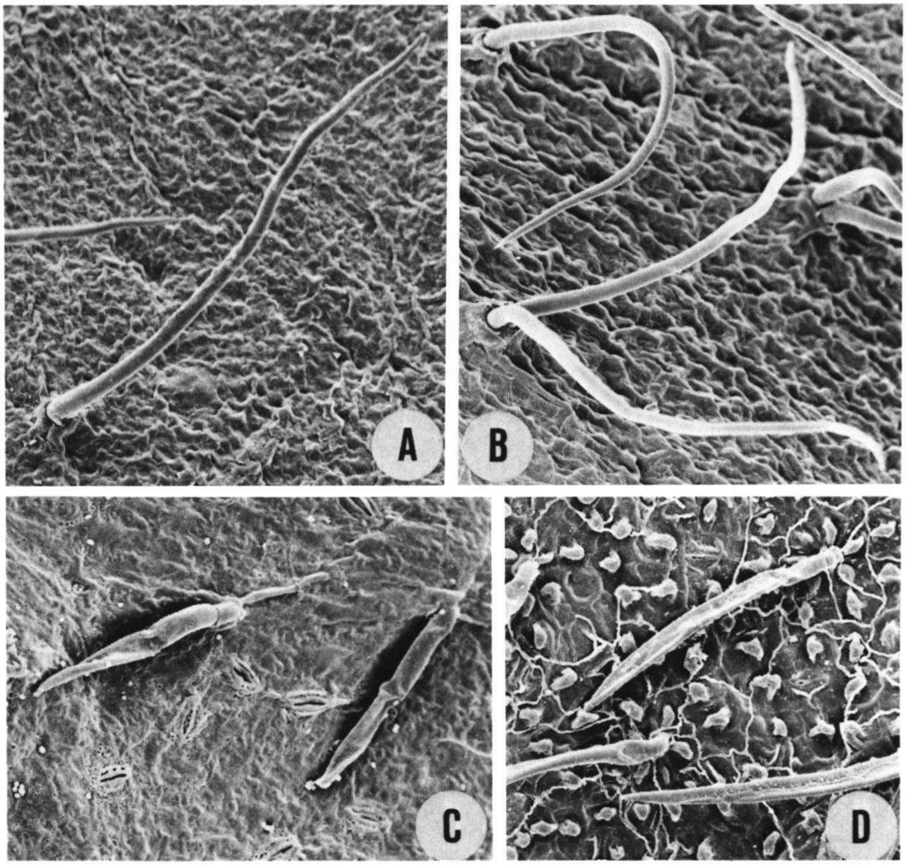


Fig. 5. Scanning electron micrographs of leaves of *Annona* species. — A: *Annona* spec. Single, long, erect trichomes on the adaxial side of the leaf; × 180. — B: *A. macrocalyx.* A tuft of two erect trichomes on the adaxial side of the leaf; × 280. — C: *A. muricata* 1. Short appressed trichomes on the abaxial side of the leaf; × 270. — D: *A. spraguei.* Short appressed trichomes. Note the distinct papillae of the abaxial epidermis; × 270.

Selected leaf anatomical characteristics

All characters studied are listed in table 5. Here the species are grouped according to the classification proposed by Fries (1931, 1959; table 4).

Lamina thickness – In table 5 three classes of lamina thickness are recognized: less than 100 µm, between 100 and 250 µm, and over 250 µm. Species of section 1 all showed thicknesses in the latter category (fig. 4A). However, most other species have a lamina thickness between 100 and 250 µm. Two specimens (*Annona diversifolia* and *A. squamosa* 3) possessed a very thin lamina.

Table 5. Leaf anatomical characters of *Annona* species.[1]

Section	species	oil		muc		lamina	
		p	s	p	s	thick	scl
1	*deminuta*	+	+	–	–	+++	++
	foetida	+	+	–	–	+++	++
	montana 1–4	+	+	–	–	+++	+
	muricata 1–4	+	+	–	–	+++	+
3	*purpurea*	–	–	–	+	++	–
6	*glabra* 1, 2	+	+	–	–	+++	–
7	*chrysophylla*	–	+	+	–	+++	–
	glauca	+	+	+	+	+++	++
	senegalensis 1	+	+	+	–	++	–
	senegalensis 2	–	–	+	+	++	–
8	*cordifolia*	+	+	+	+	++	–
	hypoglauca	+	+	–	+	++	–
	cf. *macrocalyx*	+	+	+	–	++	–
	scandens	+	+	+	–	++	–
	sericea 1	+	+	+	–	++	–
	sericea 2	+	+	+	–	++	–
	sericea 3	+	+	+	+	++	–
	spraguei	+	+	–	–	++	–
	spec.	+	+	+	+	++	–
9	*nutans*	–	+	+	–	+++	–
10	*amazonica*	+	+	–	–	++	–
	cacans	–	+	–	–	++	–
	duckei 1	–	+	–	–	++	–
	duckei 2	–	+	–	–	++	–
11	*acutiflora*	+	+	+	+	++	–
	ambotay	+	+	–	–	++	+
	hayesii	+	+	–	–	++	–
12	*cherimola* 1, 2	+	+	–	–	++	–
	longiflora	+	–	+	+	++	–
	reticulata 1, 2	+	+	+	+	++	–
	squamosa 1	+	+	+	+	++	–
	squamosa 2	+	+	+	+	++	–
	squamosa 3	+	–	+	+	+	–
	squamosa 4	+	+	+	+	++	–
13	*pittieri*	–	–	+	+	++	–
14	*diversifolia*	–	+	–	–	+	–
16	*gracilis*	+	–	+	+	++	–
	haitensis	+	+	–	–	+++	–
17	*bicolor* 1	+	+	+	–	++	–
	bicolor 2	+	+	–	–	+++	–
	dumetorum	+	+	+	+	++	–
	globiflora	–	+	–	–	++	–
—	*phaeoclados*	+	+	–	–	++	–
	tenuiflora	+	+	+	–	++	+
	spec. nov.	–	+	+	–	++	–

[1]) For legends, see page 428.

(Table 5 continued)[1]

adaxial epidermis / hairs						abaxial epidermis / hairs				
cut	hyp	cry	fr	le	typ	pa	st	fr	le	typ
–	–	–	±	+	a	–	–	±	+	a
–	–	–	±	+	a	–	–	+	+	a
–	–	–	±	+	a	–	–	+	+ (+)	a
–	–	–	–			–	–	++	+ (+)	a
–	–	–	+	++++	a	–	–	++	++++	a
–	–	–/d	–			–	–	–		
–	+	d	++	+++	a	–	–	++++	+++	e
–	+	–	+	+	a	±	–	++	++	a
+	+	–	+	++	e	–	–	+	++	a
+	+	–	++	++	e	–	–	++++	++	e
+	+	d	+	++	e	±	–	++	++	e
+	–	dd	±	+	a	–	+	+++	++	a
–	–	(d)	++ à 2	+++	e	±	–	++++ à 2	++++	e
–	–	dd	+	+++	e	–	–	++ (à 2)	++	e
–	–	dd	++ à 2	++++	e	–	–	++++ à 2	+++	e
–	–	(d)	–			–	–	+ à 2	+++	e
–	–	d	+++ à 2	++	e	–	–	+++++ à 2	+++	e
+	–	–	+	+	e	+	–	+++	++	a
+	–	(d)	+	++++	e	±	–	+	+++	e
+	+	d	–			–	+	+	+	a
+	–	–	–			+	–	+++	+	a
+	–	–	–			–	–	+	+	a
–	–	–	+	+	a	±	–	+++	+	a
–	–	–	+	+	a	±	–	++	+	a
–	–	–	+	++	a	–	–	+	++	a
–	–	–	+	++	e	–	–	++ à 2	++	e
–	–	–	++	++	a	–	–	++	++	a
–	–	–	+ (+)	+++	e	±	–	+++ (+)	++++	e
–	–	–	+++	++	e	–	–	+++	++++	e
–	–	(d)	+	++	a	–	–	+	++	a
–	–	–	–			–	–	±	++	a
–	–	–	±	++	a	–	–	++	++	a
–	–	–	+	++	a	–	–	+	++	a
–	–	–	–			–	–	±	+	a
+	–	–	+	+	a	–	–	+	++	e
–	–	r	+++	++++	e	–	–	+++++	+++	e
–	–	–	–			–	–	++	+	a
–	–	–	–			+	–	++	+++	e
–	–	(d)	±	+	a	±	–	++	+	a
–	–	–	–			–	–	±	+	a
–	–	d	–			–	–	++	+	a
–	–	–	–			–	–	++	+	a
–	+	d	–			–	–	–		
–	–	–	±	++	a	–	–	+	++	a
–	–	dd	+	++	e	±	–	+++	++ (+)	e

1) For legends, see page 428.

Explanation of abbreviations used in table 5:

Oil and mucilage (muc) cells:	p = palisade parenchyma; s = spongy parenchyma; + = present; – = absent.
Lamina thickness (thick):	+ = < 100 µm; ++ = 100–250 µm; +++ = > 250 µm.
Sclereids (scl):	+ = some sclereids; ++ = network of sclereids.
Cuticle thickness (cut):	– = ≤ 2 µm; + = > 2 µm.
Adaxial hypodermis (hyp):	– = absent; + = present.
Epidermal crystals (cry):	(d) = druses infrequent; d = druses present; dd = druses abundant; r = rhombic crystals.
Hair frequency (fr):	± = ≤ 0.1 hair/mm leaf width; + = 0.1 < fr ≤ 1 hair/mm; ++ = 1 < fr ≤ 2 hairs/mm; +++ = 2 < fr ≤ 5 hairs/mm; ++++ = 5 < fr ≤ 10 hairs/mm; +++++ = > 10 hairs/mm; (à2) = tufts of 2 hairs infrequent; à2 = tufts of 2 hairs abundant.
Hair length (le):	+ = ≤ 100 µm; ++ = 100 < le ≤ 200 µm; +++ = 200 < le ≤ 300 µm; ++++ = > 300 µm.
Type of hair (typ):	a = appressed; e = erect.
Papillate epidermis (pa):	– = flat; ± = domed periclinal epidermal walls; + = papillae.
Stomata (st):	+ = stomata sunken; – = stomata in level with epidermis.

Sclereids in mesophyll – In most species sclereids are absent in the mesophyll (table 5). In species of section 1, *Annona,* and three other species, long sparsely branched (filiform) sclereids associated with bundle sheath sclerenchyma strands were found (fig. 4A; table 5). In a few species the sclereids formed a network throughout the mesophyll, reaching from the upper to the lower epidermis (table 5; *A. deminuta, A. foetida,* and *A. glauca:* fig 4A), sometimes constituting a layer of sclereids directly underneath the upper epidermis (in *A. deminuta* and *A. foetida*).

Adaxial cuticle and epidermal wall – In most species the cuticle has a normal thickness (< 2 µm). However, in a few species the cuticle had a thickness of approximately 5 µm (fig. 3D; table 5: *A. spraguei* and *A. cacans*).

In some species (*A. foetida, A. globiflora, A. montana,* and *A. reticulata*) the outer periclinal wall of the adaxial epidermal cells was very thick (fig. 3D).

Adaxial hypodermis – In a few species a one-layered hypodermis is present (fig. 3C; table 5). All species studied of section 7, *Helogenia,* and *A. glabra* (section 6, *Phelloxylon*), *A. cordifolia, A. nutans,* and *A. phaeoclados* have this characteristic (table 5). In most cases a single-layered epidermis is present, however (fig. 3D; table 5).

Crystals in the adaxial epidermis – In many species of *Annona* druse crystals are present in the adaxial epidermis (fig. 3A; table 5). In only one species, *A. diversifolia,* rhombic (square-hexagonal, plate-like) crystals were observed (fig. 3B; table 5). Most species lacked crystals, however (table 5). The two specimens of *A. glabra* and *A. bicolor* show variations in the absence/presence of druse crystals in the epidermis.

Trichomes – Uniseriate non-glandular hairs of 2(–4) cells, including a distinct basal cell, are frequently encountered (table 5). In most cases the hairs are single (figs. 3E, 5A, C, D). However, small tufts of mostly 2 (rarely 3 to 4) simple hairs were frequently found together with single hairs on leaves of *Annona macrocalyx* (fig. 5B) and *A. sericea* (fig. 3F) and sporadically on the abaxial side of the leaf in

A. scandens and *A. ambotay* (à2 in table 5). Two different types of hairs could be recognized: appressed (figs. 3E, 5C, D) and erect (figs. 3F, 5A, B) hairs. Appressed hairs are mostly thin-walled (fig. 3E), whereas the erect hairs are thick-walled (fig. 3F). The first hair type occurs most frequently on both adaxial and abaxial sides of the leaf. The hairs vary from short (< 100 µm) to very long (> 400 µm). They were classified into length categories which showed little overlap at least on the species level (table 5).

The density of trichomes on either side of the leaf was estimated by counting the number of hairs between the veins in transverse section. In many species hairs are present on the adaxial epidermis of the leaves, ranging from very few to many (table 5). The length of the hairs varies from short (100 µm) to long (360 µm; fig. 5A, B). In 18 species no hairs are found on the adaxial epidermis.

Nearly all species studied possess hairs on the abaxial side of the leaf (table 5). Their number (which is usually higher than on the adaxial side) varied from very few to abundant (table 5). The length of the hairs on the adaxial epidermis varies from 70 µm (figs. 3E, 5A) to 500 µm. Only 2 species did not possess hairs on either side of the leaf: *Annona glabra* and *A. phaeoclados* (table 5). No species were observed in which only hairs occurred on the adaxial epidermis.

Some infraspecific variation is present. *Annona sericea* 2 only possesses a few abaxial trichomes, whereas the other specimens have many trichomes on both sides (table 5). In *A. squamosa* two of the four specimens studied lack adaxial hairs.

Papillate abaxial epidermis – In most cases the outer periclinal wall of the abaxial epidermis cells is flat (figs. 4B, 5C). In a few cases the epidermis shows distinct dome-shaped papillae (figs. 4C, 5D). In some species the epidermal cells showed slightly domed periclinal outer cell walls (table 5).

Sunken stomata in abaxial epidermis – In two species (*A. nutans, A. hypoglauca*) the abaxial epidermis is rather thick and the stomata are sunken (fig. 4D; table 5). In the other species the stomata were in level with the abaxial epidermis.

Tentative cladistic analysis

In order to investigate the value of oil and mucilage cells as phylogenetic markers tentative cladistic analyses were carried out.

The closely related genus *Rollinia* was chosen as outgroup in the first analysis. Its character states are based upon the preliminary cladistic analysis (Koek-Noorman, 1990) and leaf anatomical descriptions of *Rollinia* (Van Setten, unpublished results; Maas & Westra, 1992). For a second analysis we created a hypothetical general, and possibly more reliable, outgroup based upon the 'common is primitive' principle (Watrous & Wheeler, 1981). The character states are based on widespread leaf anatomical features in the Annonaceae (Van Setten, unpublished results). The characters and character states used in the analyses are presented in table 6a & b. Unknown character states are scored as '?'.

The first runs with either *Rollinia* or the hypothetical group as outgroup gave initial trees (command mhennig) with a consistency index (CI) of 0.25 and 0.23 respectively. Branch and bound options (command bb*) resulted in more than 2000 equally parsimonious trees for both outgroups. To select among those parsimonious trees character weights (command xsteps w) were applied.

When *Rollinia* was used as outgroup the weight factors did not alter anymore after 5 iterative character weighting procedures. The final result was 1 initial tree with an increased CI of 0.50. The branch and bound option still resulted in more than 2000 equally parsimonious trees. In figure 6 the initial tree after character weighting is presented. Many polytomies have shown up (allowed by HENNIG86). In this tree only character 10 (loss of double adaxial hairs) is placed without the assumption of homoplasy (CI = 1.0; fig. 6). The other characters all have a CI below 1.0 and thus show events of homoplasy (i.e. parallel developments and/or reversals). The idioblast characters 1, 3, and 4 show very low CI values: 0.1, 0.06, and 0.07 respectively. Character 2 (oil cells in spongy parenchyma), however, has a CI of 0.5. The tree is mainly based on mucilage cells, epidermal druses, and number and type of

Table 6a. Character states of the leaf anatomical characters used in the cladistic analyses using HENNIG86.

Characters

1. Oil cells in palisade parenchyma
   1. absent
   2. present
2. Oil cells in spongy parenchyma
   1. absent
   2. present
3. Mucilage cells in palisade parenchyma
   1. absent
   2. present
4. Mucilage cells in spongy parenchyma
   1. absent
   2. present
5. Lamina thickness
   1. < 100 μm
   2. 100–250 μm
   3. > 250 μm
6. Sclereids in mesophyll
   1. absent
   2. present
7. Crystals in adaxial epidermis
   1. absent
   2. druses present
   3. rhombic crystals present
   4. druses and rhombic crystals present
8. Hypodermis
   1. absent
   2. present
9. Number of adaxial hairs
   1. absent
   2. ≤ 1 hair/mm leaf width
   3. 1 < n ≤ 5 hairs/mm leaf width
   4. > 5 hairs/mm leaf width

Characters

10. Implantation of hairs adaxially
    1. single
    2. single and double hairs
11. Type of adaxial hairs
    1. appressed
    2. erect
    3. appressed and erect
12. Abaxial epidermis
    1. flat
    2. domed periclinal outer wall
    3. papillae
13. Stomata in abaxial epidermis
    1. in level with outer epidermal walls
    2. sunken in epidermis
14. Number of abaxial hairs
    1. absent
    2. ≤ 1 hair/mm leaf width
    3. 1 < n ≤ 5 hairs/mm leaf width
    4. > 5 hairs/mm leaf width
15. Implantation of abaxial hairs
    1. single
    2. single and double hairs
16. Type of abaxial hairs
    1. appressed
    2. erect
    3. appressed and erect
17. Thickness of adaxial cuticle
    1. absent
    2. ≤ 2 μm
    3. > 2 μm

Table 6b. Datamatrix for cladistic analyses of leaf anatomical characters using HENNIG86, with either *Rollinia* or a created general annonaceous outgroup as the outgroup.

Species / character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Rollinia	2	2	1	1	2	1	4	1	3	2	3	1	1	3	2	3	1
general outgroup	1	2	1	1	2	1	2	1	1	?	?	1	1	3	1	2	2
Annona																	
acutiflora	2	2	2	2	2	1	1	1	2	1	1	1	1	2	1	1	2
amazonica	2	2	1	1	2	1	1	1	1	?	?	3	1	3	1	1	3
ambotay	2	2	1	1	2	2	1	1	2	1	2	1	1	3	2	1	2
bicolor 1	2	2	2	1	2	1	2	1	2	1	1	2	1	3	1	1	2
bicolor 2	2	2	1	1	3	1	1	1	1	?	?	1	1	2	1	1	2
cacans	1	2	1	1	2	1	1	1	1	?	?	1	1	2	1	1	3
cherimola 1, 2	2	2	1	1	2	1	1	1	3	1	2	2	1	4	1	2	2
chrysophylla	1	2	2	1	3	1	2	2	3	1	1	1	1	4	1	2	2
cordifolia	2	2	2	2	2	1	2	2	2	1	2	2	1	3	1	2	2
deminuta	2	2	1	1	3	2	1	1	2	1	1	1	1	2	1	1	2
diversifolia	1	2	1	1	1	1	3	1	3	1	2	1	1	4	1	2	2
duckei 1	1	2	1	1	2	1	1	1	2	1	1	2	1	3	1	1	2
duckei 2	1	2	1	1	2	1	1	1	2	1	1	2	1	2	1	1	2
dumetorum	2	2	2	2	2	1	2	1	1	?	?	1	1	3	1	1	2
foetida	2	2	1	1	3	2	1	1	2	1	1	1	1	2	1	1	3
glabra 1	2	2	1	1	3	1	1	1	1	?	?	1	1	1	?	?	2
glabra 2	2	2	1	1	3	1	2	1	1	?	?	1	1	1	?	?	2
glauca	2	2	2	2	3	2	1	2	2	1	1	3	1	3	1	1	2
globiflora	1	2	1	1	2	1	1	1	1	?	?	1	1	3	1	1	2
gracilis	2	1	2	2	2	1	1	1	1	?	?	1	1	3	1	1	2
haitiensis	2	2	1	1	3	1	1	1	1	?	?	3	1	3	1	2	2
hayesii	2	2	1	1	2	1	1	1	3	1	1	1	1	3	1	1	2
hypoglauca	2	2	1	2	2	1	2	1	2	1	1	1	2	3	1	1	2
longiflora	2	1	2	2	2	1	1	1	3	1	2	1	1	3	1	2	2
cf. *macrocalyx*	2	2	2	1	2	1	2	1	3	2	2	2	1	4	2	2	2
montana 1–4	2	2	1	1	3	2	1	1	2	1	1	1	1	2	1	1	2
muricata 1–4	2	2	1	1	3	2	1	1	1	?	?	1	1	3	1	1	2
nutans	1	2	2	1	3	1	2	2	1	?	?	1	2	2	1	1	3
phaeoclados	2	2	1	1	2	1	2	2	1	?	?	1	1	1	?	?	2
pittieri	1	1	2	2	2	1	1	1	2	1	1	1	1	2	1	2	3
purpurea	1	1	1	2	2	1	1	1	2	1	1	1	1	3	1	1	2
reticulata 1, 2	2	2	2	2	2	1	2	1	2	1	1	1	1	2	1	1	2
scandens	2	2	2	1	2	1	2	1	2	1	2	1	1	3	2	2	2
senegalensis 1	2	2	2	1	2	1	1	2	2	1	2	1	1	2	1	1	3
senegalensis 2	1	1	2	2	2	1	1	2	3	1	2	1	1	4	1	2	3
sericea 1	2	2	2	1	2	1	2	1	3	2	2	1	1	4	2	2	2
sericea 2	2	2	2	1	2	1	2	1	1	?	?	1	1	2	2	2	2
sericea 3	2	2	2	2	2	1	2	1	3	2	2	1	1	4	2	2	2
spec.	2	2	2	2	2	1	2	1	2	1	2	2	1	2	1	2	3
spec. nov.	1	2	2	1	2	1	2	1	2	1	2	2	1	3	1	2	2
spraguei	2	2	1	1	2	1	1	1	2	1	2	3	1	3	1	1	3
squamosa 1	2	2	2	2	2	1	1	1	1	?	?	1	1	2	1	1	2
squamosa 2	2	2	2	2	2	1	1	1	2	1	1	1	1	2	1	1	2
squamosa 3	2	1	2	2	1	1	1	1	2	1	1	1	1	2	1	1	2
squamosa 4	2	2	2	2	2	1	1	1	1	?	?	1	1	2	1	1	2
tenuiflora	2	2	2	1	2	2	1	1	2	1	1	1	1	2	1	1	2

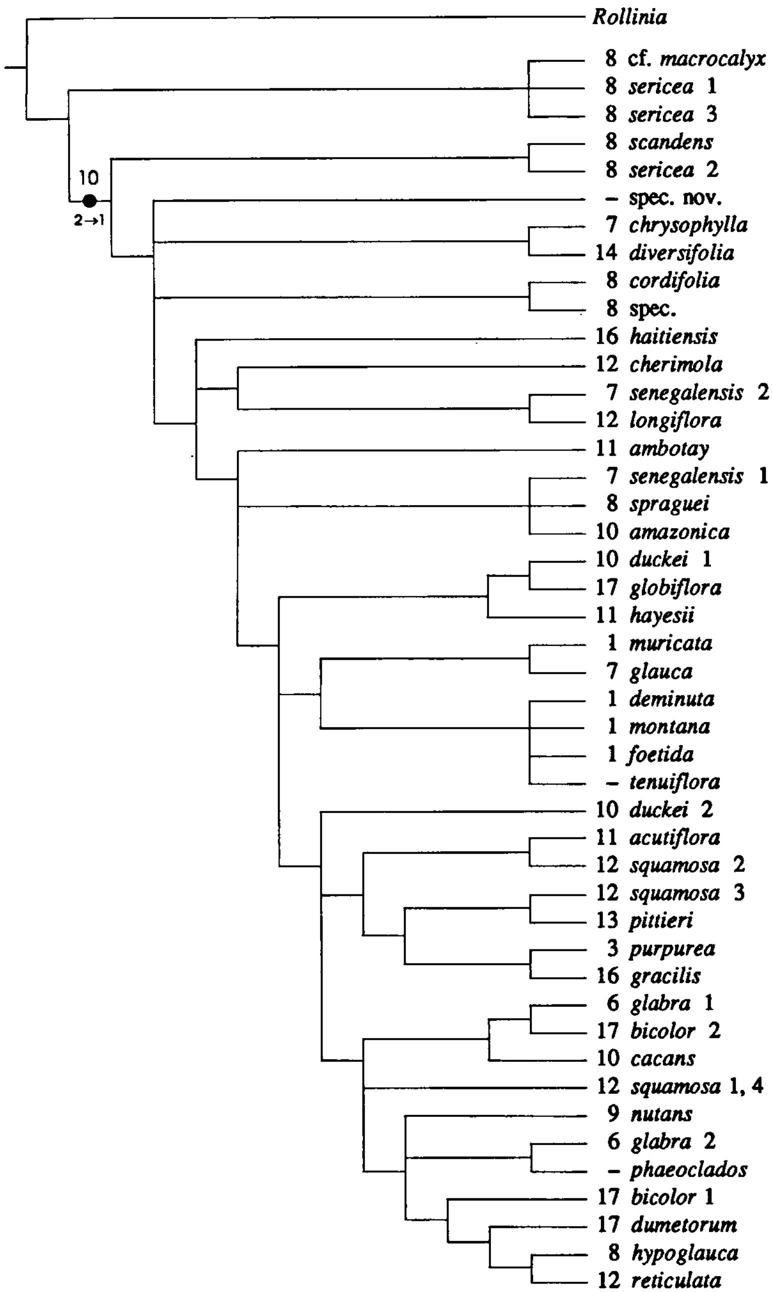


Fig. 6. The initial cladogram with *Rollinia* as outgroup resulting from the phylogenetic analysis using all leaf anatomical characters after five character weighting procedures. Only character 10 is placed on the tree as an apomorphy (loss of double hairs on the adaxial side of the leaf). The numbers placed in front of the species names represent the sections in the current classification of *Annona*.

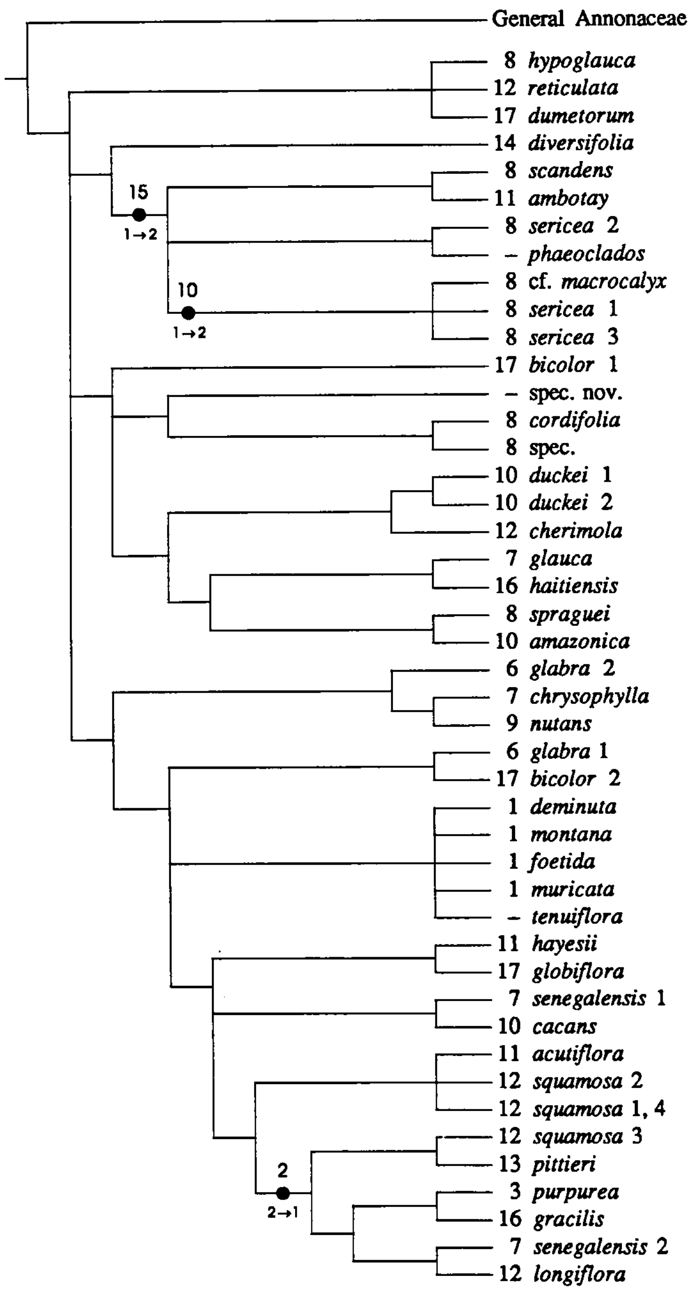


Fig. 7. The initial cladogram with a hypothetical general annonaceous outgroup resulting from the phylogenetic analysis using all leaf anatomical characters after six iterative character weighting procedures. Three characters are placed on the tree as an apomorphy (character 15, 10 [presence of double hairs on the adaxial and abaxial side of the leaf] and 2 [loss of oil cells in the spongy parenchyma]). Numbers refer to sections.

adaxial and abaxial hairs. Species of section 1 are grouped together in the middle part of the tree, based on the presence of sclereids (fig. 6). Further, some species of section 8 are placed near each other and the outgroup in the tree (based on the presence of tufts of hairs).

For the hypothetical general outgroup the weight factors stabilized after 6 iterative character weighting procedures. The result was still more than 2000 equally parsimonious trees with an increased CI of 0.55. The final initial tree is depicted in figure 7. In this tree three characters had a CI of 1.0: character 2 (loss of oil cells in spongy parenchyma) and characters 10 and 15 (presence of double hairs on adaxial and abaxial side respectively). Again the remaining idioblast characters had low CI values: 0.1, 0.08, and 0.2 respectively. The tree is mainly based on mucilage cells, epidermal druses, and number of adaxial and abaxial hairs. In this cladogram the species of section 1 once more are closely related, based on the presence of sclereids.

DISCUSSION

General remarks

In some earlier studies on leaf anatomical characters of Annonaceae oil and mucilage cells are classified as 'oil cells' (Beyer, 1902; Van Setten & Koek-Noorman, 1986) or as secretory cells (Jovet-Ast, 1942). West (1969), however, made the distinction between oil and mucilage cells based upon their morphology. In the present paper we distinguished between oil and mucilage cells, based on the different morphological appearances of the idioblasts after specific staining procedures at the light microscopical and ultrastructural level (Bakker & Gerritsen, 1990; Bakker et al., 1991).

Most leaf anatomical features discussed in the present paper were already described by other investigators: sclereids in the mesophyll (Blenk, 1884; Rao & Chin, 1966), epidermal crystals (Van der Wijk, 1950; Maas & Westra, 1992), adaxial hypodermis (described as two-layered or multiple epidermis) and papillate abaxial epidermis (Beyer, 1902; Robyns & Ghesquière, 1934; Jovet-Ast, 1942; Van Setten & Koek-Noorman, 1986). In the absence of ontogenetical information the term hypodermis is preferred over the terms two-layered epidermis and multiple epidermis, as used by Beyer (1902), Jovet-Ast (1942), Roth (1981), and Van Setten & Koek-Noorman (1986). Roth (1981) stated that most of these features had little taxonomic value for the Annonaceae (family level). The uniseriate, non-glandular hair type has been reported by several authors (Beyer, 1902; Van der Wijk, 1950; Aleykutty & Inamdar, 1980; Van Setten & Koek-Noorman, 1986). It is common throughout the Annonaceae and probably primitive for the family (Roth, 1981).

Comparison with Fries' classification

When comparing the distribution of oil and/or mucilage cells in the mesophyll and other leaf anatomical features (table 5) with the current classification, it should be realized that the number of specimens and species studied is too limited to determine the full variation at species and section level. Yet some patterns are apparent. Section 1 is the only section containing more than one species of which all species studied exclusively possess oil cells in the palisade and the spongy parenchyma. Section 6 with *Annona glabra* as only species also exclusively showed oil cells in both layers of the

mesophyll (table 5). This is in agreement with the former inclusion of this species in Section 1 (Safford, 1911). The other sections are heterogeneous for the distribution patterns of oil and/or mucilage cells (table 5). Despite this heterogeneity the distribution patterns of the idioblasts could be profitably used in identification of some specimens, e.g. one specimen (table 1: *Berg & Akkermans 1058*) was determined as *A. duckei*; another specimen (table 1: *Maas et al. 6186*) was included in section 8 (P. Maas, pers. comm.).

The great infrageneric and in some cases even infraspecific variation in distribution patterns of idioblasts (e.g. in *A. bicolor, A. sericea,* and *A. squamosa*; table 5) can be understood in the light of the postulated homology of oil and mucilage cells (Bakker et al., 1991). Apparently small genotypic or perhaps even phenotypic modification can alter the distribution pattern of idioblasts in leaves of *Annona*. Ontogenetic and seasonal variations have been reported to affect the amount of oil produced by a plant (Shiva & Jain, 1987) and even the number of oil and mucilage cells in leaves (Shirasawa, 1903).

The frequencies of the idioblasts show some variation but are more or less diagnostic for sections/species, e.g. species of sections 7 and 8 mostly possess high numbers of both idioblast types (compare tables 2 and 5).

In combination with oil/mucilage distribution patterns, the additionally recorded leaf anatomical features (table 5) in some cases allow a more or less diagnostic characterization of individual sections.

A thick lamina, the presence of sclereids in the mesophyll, short appressed abaxial hairs in relatively low numbers, a flat epidermis, and the presence of oil cells in both mesophyll layers are characteristic for section 1, *Annona.*

Section 6, *Phelloxylon,* is characterized by the absence of hairs and by the presence of oil cells in both mesophyll layers.

Adaxial and abaxial hairs, an adaxial hypodermis, and the presence of mucilage cells in the palisade parenchyma are characteristic for (a subsection of) section 7, *Helogenia.*

Presence of druses in the adaxial epidermis are typical for section 8, *Pilannona,* together with hairs on both the adaxial and abaxial side and the presence of oil cells in both mesophyll layers. In most species also mucilage cells are present. Two species were characterized by the abundance of small tufts of hairs.

Species in section 10, *Oligantha,* always possess short appressed abaxial hairs and exclusively oil cells in the mesophyll.

Species in section 11, *Atractanthus,* always possess hairs of medium length on both sides of the leaf and oil cells are present in both mesophyll layers.

In section 17, *Annonella,* short appressed abaxial hairs are present together with oil cells in spongy parenchyma.

It should be emphasized that the above leaf anatomical characterizations of most sections are very weak, and that usually some species or specimens are exceptional in the presence/absence of one or more leaf anatomical features. Leaf anatomical character associations, including those of oil and mucilage cells, are in fact only constant in section 1, *Annona.* If we assume that Fries' classification is a natural one, this implies that the systematic and diagnostic value of most leaf anatomical features is quite limited at the sectional level in *Annona.*

Tentative cladistic analysis

The computer program HENNIG86 proved to be the best program for phylogenetic analysis (Van Welzen, 1989). It was not the purpose of the present study to present a complete description of the leaf anatomical characters within the genus *Annona*. Instead we wished to explore the variation in idioblast distribution throughout the genus in correlation with (some) other leaf anatomical features. The cladistic analyses above presented are applied to get an impression of the possible value of the idioblasts as phylogenetic markers. More detailed and extended studies are needed to enlarge the number of characters, species and specimens to find better resolved trees.

For a phylogenetic analysis the group under study has to be monophyletic. The genus *Annona* is very homogeneous in its flower and fruit morphological features (Fries, 1931, 1943, 1959) and pollen morphology (Walker, 1971). Together with the genera *Rollinia* and *Raimondia*, *Annona* forms the Annona-group sensu Fries, which is considered to be very natural. However, it is not known which of the features characterizing the genus *Annona* represent plesiomorphic or apomorphic traits in this group.

In this study we tested two different outgroups, since the 'real' ancestor of the genus *Annona* is still unknown. The first outgroup used, *Rollinia*, is thought to be derived from *Annona* (Fries, 1943). This would invalidate the first analysis, because some shared characters interpreted as symplesiomorphies by the HENNIG86 program are probably synapomorphies. In addition, a cladistic analysis of *Rollinia* (Koek-Noorman, 1990) showed similar results as for *Annona* in the present study. For both genera satisfactory cladograms were lacking. In the second analysis a hopefully more reliable character polarization has been achieved by the use of the admittedly controversial commonality principle (Watrous & Wheeler, 1981). Moreover, more detailed studies on the leaf anatomy of *Annona* and other annonaceous genera are needed to reconfirm the hypothesized character states for the outgoup.

The fact that after character weighting in both analyses still more than 2000 trees were generated shows that the leaf anatomical features do not support the distribution of the same groups nor corroborate each other; many homoplasies exist.

The generally low consistency indices of the idioblast characters in both analyses indicate many parallel origins or losses of an idioblast type in the palisade or spongy tissue. This strongly reduces the value of oil and mucilage cells as systematic or phylogenetic markers. As mentioned in the comparison with Fries' classification this can be understood in the light of the homologous nature of oil and mucilage cells (Bakker et al., 1991).

CONCLUSIONS

Oil and/or mucilage cells are always present in the leaves of *Annona*, at least in the spongy parenchyma. The distribution, combinations and frequencies in which the idioblast types appear, is of limited diagnostic value at the species level and in some cases indicates affinity between related species (e.g. species of section 1, *Annona*). Tentative cladistic analyses indicate, however, that the distribution of various character states in species of *Annona* is due to many homoplaseous events (parallel origins and reversals), which restricts the value of these characters as phylogenetic markers. This can be (partly) understood in the light of the hypothesized homology of oil and mucilage cells.

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